Vaccine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

The generation of a simian adenoviral vectored HCV vaccine encoding genetically conserved gene segments to target multiple HCV genotypes

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ARTICLE INFO

Article history:
Received 6 April 2017
Received in revised form 29 September 2017
Accepted 26 October 2017
Available online xxxx

Keywords:
T cell
HCV vaccine
Conserved segments
Cross-reactivity
Simian adenovirus

ABSTRACT

Background: Hepatitis C virus (HCV) genomic variability is a major challenge to the generation of a prophylactic vaccine. We have previously shown that HCV specific T-cell responses induced by a potent T-cell vaccine encoding a single strain subtype-1b immunogen target epitopes dominant in natural infection. However, corresponding viral regions are highly variable at a population level, with a reduction in T-cell reactivity to these variants. We therefore designed and manufactured second generation simian adenovirus vaccines encoding genomic segments, conserved between viral genotypes and assessed these for immunogenicity.

Methods: We developed a computer algorithm to identify HCV genomic regions that were conserved between viral subtypes. Conserved segments below a pre-defined diversity threshold spanning the entire HCV genome were combined to create novel immunogens (1000–1500 amino-acids), covering variation in HCV subtypes 1a and 1b, genotypes 1 and 3, and genotypes 1–6 inclusive. Simian adenoviral vaccine vectors (ChAdOx) encoding HCV conserved immunogens were constructed. Immunogenicity was evaluated in C57BL6 mice using panels of genotype-specific peptide pools in *ex-vivo* IFN-ϒ ELISpot and intracellular cytokine assays.

Results: ChAdOx1 conserved segment HCV vaccines primed high-magnitude, broad, cross-reactive T-cell responses; the mean magnitude of total HCV specific T-cell responses was 1174 SFU/10^6 splenocytes for ChAdOx1-GT1-6 in C57BL6 mice targeting multiple genomic regions, with mean responses of 935, 1474 and 1112 SFU/10^6 against genotype 1a, 1b and 3a peptide panels, respectively. Functional assays demonstrated IFNg and TNFa production by vaccine-induced CD4 and CD8 T-cells. *In silico* analysis shows that conserved immunogens contain multiple epitopes, with many described in natural HCV infection, predicting immunogenicity in humans.

Conclusions: Simian adenoviral vectored vaccines encoding genetic segments that are conserved between all major HCV genotypes contain multiple T-cell epitopes and are highly immunogenic in pre-clinical models. These studies pave the way for the assessment of multi-genotypic HCV T-cell vaccines in humans.

1. Introduction

Hepatitis C virus (HCV) infects approximately 170 million people worldwide and is a leading cause of end-stage liver disease. HCV treatment has become highly effective in the era of directly acting antivirals (DAA), with the latest drugs showing high efficacy against all HCV genotypes [1,2]. However, the new drugs are expensive [3] and treatment rates remain low ranging from 3.5% in Europe to 21% in the US [4,5]. Furthermore, successful treatment does not prevent re-infection, a particular problem in intra-venous drug using populations [6,7]. Notably, a recent World Health Organization (WHO) report shows that even with effective treatment, available new HCV infections in 2015 exceeded the number of people dying of the disease or receiving curative therapy, leading to an increase in HCV prevalence [8]. Therefore, a HCV vaccine remains an important goal.

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https://doi.org/10.1016/j.vaccine.2017.10.079
0264-410X/

A major challenge for HCV vaccine development is the extensive viral variability of HCV that exists as seven major genotypes globally that are approximately 80% genetically homologous, and numerous HCV subtypes [9]. Whilst HCV genotype-1 is the most common genotype world wide, other genotypes are distributed in areas with high HCV prevalence with genotype-2 common in Asia, genotype-3 in the UK, Asia and former USSR, genotype-4, 5 and 7 in Africa, and genotype-6 in Asia [9]. In addition, HCV exists as a viral population of closely related genetic variants (quasispecies) within the infected host. Immunodominant T-cell responses in natural HCV infection are known to focus on a small number of epitopes that often show high sequence variability [10]. However, T-cell specificity is distinct between HCV genotypes [11] with limited T-cell cross-reactivity against common sequence variants at frequently detected T-cell targets [12].

We have previously shown that HCV vaccine strategies using simian adenovectors encoding HCV genotype-1b non-structural regions induce high magnitudes of HCV specific T-cells at epitopes dominant in natural infection [13,14]. However, we observed reduced T-cell cross-reactivity of vaccine-induced T-cells against other genotypes [13] and against circulating viral variants at epitopes derived from the same genotype [15]. An effective global T-cell vaccine will need to generate T-cell responses capable of recognising viral variant epitopes between both quasispecies, and between people infected with different genotypes.

In this study, we introduce an immunogen design approach based on the selection of HCV genomic regions that are highly conserved between HCV genotypes with the exclusion of variable HCV epitopes [16]. We hypothesise that conserved HCV sequences carry a detrimental fitness cost if mutations occur in these regions. Therefore vaccine induced T-cells responses to conserved viral segments that are cross-reactive against multiple HCV genotypes will be capable of controlling and eliminating the virus during primary infection as viral mutation to escape T-cell recognition will carry a significant fitness cost to the virus.

We used world-wide HCV prevalence data [9] to inform the rational design of three distinct HCV immunogens targeting (i) genotype-1 alone - the most prevalent genotype globally (GT1), (ii) genotypes-1 and 3 - the two most commonly infecting genotypes in the UK [17] and world wide (GT1/3); and (iii) genotypes-1 to 6 - covering all common HCV genotypes globally (GT1-6) [18]. Novel conserved immunogens were encoded in a simian adenoviral vector ChAdOx1 [19], known to be capable of inducing high magnitude T-cell responses, which also circumvents the issue of pre-existing immunity to adenoviral vectors that may limit vaccine efficacy.

We assessed designed antigens for immunogenicity using HLA epitope prediction programs, through the analysis of epitopes described in natural HCV infection and finally through evaluating vaccine immunogenicity *in vivo* in pre-clinical mouse studies.

2. Methods

2.1. The selection of HCV sequences

Full-length genotype HCV sequences from the HCV sequence database www.hcv.lanl.gov, and in-house genotype 3 sequences were used for immunogen design (n = 216, Supplementary Table S1). Three different sequence datasets were generated: (a) HCV genotype-1 (GT1) n = 96, (b) HCV genotypes 1 and 3 (GT1/3) n = 72 and (c) HCV genotypes 1–6 (GT1-6) n = 216. Sequences from different research groups and countries were selected for each strain and manually checked for human origin. HCV subtypes were chosen according to common circulating viral strains [18]. Genotype-7 was not included as only one sequence has been published [18].

2.2. Definition of conserved HCV viral segments

Sequence diversity (normalized raw diversity, NDR) was calculated using pairwise Hamming distance. A sliding window (W) of 20 amino-acid (AA) starting at position zero and advancing one AA at a time was used to measure Hamming distance between all pairs of sequences in the alignment. A window size of 20 AA was selected to contain potential CD4 and CD8 epitopes (8–12 AA and 11–16 AA respectively (Supplementary Fig. S1). To define conserved segments in the dataset, we selected a threshold equal to the lowest quartile (denoted Θ) of the overall diversity distribution.

2.3. Selection of a circulating isolate for final immunogen design

A consensus sequence was generated for each dataset (GT1, GT1/3 and GT1-6); hereafter named "overall consensus", and all subtypes (e.g. for genotype-1 and genotype-3 in the GT1/3 analysis); hereafter named "subtype consensus". A circulating HCV isolate from a subtype within the dataset, with a highest homology to the overall consensus was selected and included in conserved immunogens.

2.4. Prediction analysis for T- and B-cell epitopes and proteasomal cleavage sites

Prediction of proteasomal cleavage sites was run using server NetChop 3.1, (version C-term 3.0). Epitope prediction analysis was run using online epitope prediction programs NetMHC, Syfpeithi and BIMAS with a cut-off for strong epitope binders of <0.5, >20, and >100, respectively. Potential immunogenicity of HCV immunogens was evaluated using the NetMHC prediction server (v3.4) for T-cell epitopes. B-cell epitopes were predicted with BepiPred 2.0 using a cut-off for strong binding epitopes 0.55 and an epitope prediction length of 5–20 AAs. Junctions between two concatenated conserved segments were assessed for strong binders using two independent prediction algorithms for HLA class-I and class-II types at HLA population frequency over 2% (NCBI database). Predicted strong binders were abrogated using linkers consisting of 2–6 AA (glycine/proline or glycine/serine combinations) as previously described [20,21]. A blast analysis was performed (https://blast.ncbi.nlm.nih.gov) to ensure there was no homology with the human genome at junctional regions.

2.5. Analysis of HCV T-cell epitopes in natural infection

T-cell epitopes/targets were mapped along the HCV genome to assess the association of these with genomic variability. HCV genotype-1 and genotype-3 epitopes were obtained from the immune epitope database resource (IEDB) and from those experimentally defined in our laboratory [12]. Epitopes were cross-checked with primary publications and duplications, epitope variants and epitopes described in non-human organisms were excluded.

2.6. Vaccine production

Conserved HCV immunogens were synthetically produced using "humanized" amino acid codons (GeneArt, ThermoFisher Scientific) [22] and cloned into a pENTR4 vector [19,23]. Coding cassettes within the pENTR4 vector were cloned into ChAdOx1 plasmids (Thermo Fisher Scientific LR gateway cloning procedure) and then linearized with PmeI and transfected into T-REx™-293

Please cite this article in press as: von Delft A et al. The generation of a simian adenoviral vectored HCV vaccine encoding genetically conserved gene segments to target multiple HCV genotypes. Vaccine (2017), https://doi.org/10.1016/j.vaccine.2017.10.079